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# Development of Topical Preparation for the Treatment of Psoriasis

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**ABSTRACT**

In the present research work, a sterile, thermo-sensitive, biological stable, Topical-gel capable of releasing of MTX drug at a controlled rate was formulated. The MTX drug release rate was followed non-fickian diffusion and was concentration dependant; affected by the character and ratio of polymer and copolymers. A removal in inflammatory situation and no signs of articular destruction were found in test when compared with control group throughout experimental studies. The prepared MTX topical gels proved to be capable topical drug delivery system which can decrease the dose, dosing frequency, and envisaged as an efficient topical drug delivery for the management of topical disease The best choice of precise agents and combinations needs close cooperation with the patient, always keeping in mind the inconvenient effects of the treatments. There is no single perfect combination or sequence of agents, but treatment should be set aside as simple as possible. The monotherapy is chosen, but combination therapy is the average. First-line treatment for psoriasis comprises topical corticosteroids and topical vitamin D3 analogs (either as monotherapy or in combinationThis results in hydrophobic interactions within polyoxypropylene domains and leads to the formation of gel. The gelation time for MTX gels was between 28 ± 2 seconds and 55 ± 2 seconds. As observed, gelation time decreased as the concentration of the co-polymers was increased in the formulation. Longest gelation time of 55±2 in second was recorded with F1, whereas shortest gelation time of 30±2seconds was with F8. A decrease of seconds was observed with increase of polymer concentration from 0.3% to 0.9% in formulations F3 to F8. Quick topical gels help in retaining the drug at the site of injection and release in a controlled manner.

## 1. INTRODUCTION

- Psoriasis disease is type of inflammatory disease that manifests most commonly as well-circumscribed,erythematous papules and plaques covered with silvery scales. Numerous factors are responsible such asincluding genetics. Psoriasis disease lesions are either asymptomatic or pruritic and are chiefly often local seen on the scalp,extensor surfaces of the elbows and knees, sacrum, buttocks (usually the gluteal cleft), and genitals. Thenails, eyebrows, axillae, umbilicus, and perianal area may also be affected. The disease can be common,relating confluent areas of skin extending between these regions. Lesions differ in appearance depending on type. Among the various psoriasis subtypes, plaque psoriasis (psoriasis vulgaris or chronic plaquepsoriasis) accounts or about 90%; lesions are discrete, erythematous papules or plaques covered with thick,silvery, shiny scales. Lesions appear gradually and remit and recur spontaneously or with the appearance and resolution of triggers. Psoriasis is rarely life- threatening but can affect a patient‟s self-image. Besides the patient's appearance, the sheer amount of time required to treat extensive skin or scalp lesions and to maintain clothing and bedding may adversely affect quality f life.
- **Topical treatments:** The corticosteroids are typically used external and may be injected into small or recalcitrant lesions. Externally corticosteroids are used two times daily. In-addition that is mainly efficient when used overnight under occlusive polyethylene coverings or incorporated into tape; a corticosteroid cream is useful without occlusion during the day. As lesions abate, the corticosteroid should be useful less regularly or at a lesser potency to reduce local atrophy, striae formation, and telangiectases. Externally corticosteroid use can be luxurious because great quantities (about 1 oz or 30 g) are needed for each application when a large body surface area is affected. External corticosteroids used for long period to large areas of the body may cause systemic effects and exacerbate psoriasis. For small, thick, localized, or recalcitrant lesions, high-potency corticosteroids are used with an occlusive dressing or flurandrenolide tape; these dressings are left on overnight and changed in the morning. Relapse after topical corticosteroids are stopped is often faster than with other agents
- The Vitamin D3 derivates such as calcipotriol and calcitriol, that make usual keratinocyte proliferation and differentiation; they can be used alone or in mixture with topical corticosteroids. Calcineurin inhibitors such as tacrolimus, pimecrolimus. These are obtainable in external form and are usually welltolerated. They are not as efficient as corticosteroids but may avoid the complications of corticosteroids when treating facial and intertriginous psoriasis disease. It is not clear whether they amplify the risk of lymphoma and skin cancer. Tazarotene is a topical retinoid drug. It is fewer efficient than corticosteroids as monotherapy but is a useful adjunct. Other adjunctive external treatments comprise emollients, salicylic acid, coal tar, and anthralin. The emollients contain emollient creams, ointments, petrolatum, paraffin, and even hydrogenated vegetable (cooking) oils etc. They decrease scaling and are most valuable when applied two times daily and without delay after bathing. Lesions may come out redder as scaling decreases or becomes extra transparent. These emollients are secure and should almost certainly always be used for mild to moderate plaque psoriasis

## 2.MATERIALS AND METHOD :

Methotrexate BP was received from US Amino Private Limited, Fatehnagar, Udaipur; Carbopol-934, Tamarind seed gum, Benzalkonium Chloride, Potassium Dihydrogen Phosphate, Hydrochloric Acid, Sodium Hydroxide, Dialysis Membrane 135,were analytical grade chemical and reagents procured and used.

**Preformulation study of Methotrexate:**

Physical examination of drug:

The preformulation studies of drug was carried out by physical examination i.e., colour, texture, odour etc.

**Melting point:**

Melting point of methotrexate was determined by melting point apparatus.

**Preparation of Standard Curve:**

A standard calibration curve was used to calculate the concentration of the dug penetrated through rat skin. To construct the calibration curve, stock solution was prepared by dissolving 10 mg of methotrexate in 100 ml of ethanolic phosphate buffer pH 7.4. The mixture was placed in a sonicator to ensure solubilization of the drug. Then the dilutions of 10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml were made and analyzed spectrophotometrically by using UV spectrophotometer.

**UV Analysis:**

1. 100 mg of drug was dissolved in small quantity of Phosphate buffer saline in 7.4 pH and the volume was made up to 100 ml with Phosphate buffer saline 7.4 pH. Then 1 ml of this stock solution was pipette out into a 10 ml volumetric flask and volume made up to the mark with Phosphate buffer saline 7.4 pH.

2. This resulted in preparation of stock solution of 100µg/ml. This is taken in aliquots of 2 ml, 4.0 ml, 6 ml…. up to 20 ml in to a series of 10 ml volumetric flasks of same specification and volume was made up to the mark with Phosphate buffer saline 7.4 pH.

3. The solutions are filtered through Whatmann filter paper and filtrate analysed at λ-max 303 nm using UV/visible spectrophotometer. The standard curve is plotted between absorbance and concentration.

**Distribution coefficient:**

The distribution behavior of drug is examined in n-octanol: water and n-octanol: PBS (pH 7.4) systems. It was determined by taking drug in two separating funnels containing 10 ml portions of each n-octanol and 10 ml water and 10 ml of PBS (pH 7.4). The separating funnels were shaken for 24 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically at 267 nm after appropriate dilution. The partition coefficient of the drug in phases is calculated by using formula¨s given bellow.

**Compatibility studies:**

Fourier transformed infrared (FTIR) spectra technique has been used to study the physical and chemical interaction between drug and excipients used in formulation of topical gel base.

Thin layer chromatography:

The specified amount of drug and topical gel bases were weighed separately and mixed properly with the help of spatula. The compatibility of drugs with topical gel bases was studied by thin layer chromatography by using solvent system citrate phosphate buffer pH 6.0: methanol: 70: 30.

**Preparation of novel topical-gel:**

Preparation of Methotrexate topical gel:

Topical gels of MTX were prepared by using Cold technique. MTX topical gels were formulated by using PF-127 as thermo sensitive polymer and tamarind gum of concentration, 0.3%–0.9%, was included in the formulation as co- polymer. Tamarind gum was dissolved in distilled water at 60°C and allowed to cool to room temperature. In- addition, it was carried after the solution attained 25°C ± 2°C. Accurate quantity of PF-127 was gradually added to the cold solution (5°C–10°C) of tamarind seed gum under magnetic stirring. The container was preserved and left overnight in a refrigerator at 5°C until a clear solution was obtained.

MTX was dissolved in 0.1-N NaOH and added drop wise to the preformed gel under nonstop manual stirring, to obtain the final concentration. The pH of the final formulation was adjusted to neutral by using dilute solution of Triethanolamine. Benzalkonium Chloride (0.001% w/v) was used as a preservative. The formulated gels were transferred in to 10-ml borosil glass type vials and sealed. They were stored in refrigerated at 2°C-8°C until further use.

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**Table 1.Composition of topical-gel formulation**

| FORMULATION CODE | INGREDIENTS(%) | | |
|---|---|---|---|
| | MTX | Poloxamer-407 | Tamarindgum |
| F1 | 0.5g | 20 | 0.3 |
| F2 | 0.5g | 20 | 0.6 |
| F3 | 0.5g | 20 | 0.9 |
| F4 | 0.5g | 22 | 0.3 |
| F5 | 0.5g | 22 | 0.6 |
| F6 | 0.5g | 22 | 0.7 |
| F7 | 0.5g | 23 | 0.8 |
| F8 | 0.5g | 24 | 0.9 |

Sterilization of Methotrexate topical gels: The sterilization of the optimized formulations taken and filled in glass vials, and it is sealed in a nitrogen atmosphere. Then it was carried out by using autoclave techniques

## 3.Evaluations:

**Characterization of Methotrexate gel:**

**Physical Evaluation:**

The gel was tested for physical appearance, clarity, wash ability and organoleptic characteristics by visual observation.

**Homogeneity:**

All developed methotrexate gel formulations were characterized for homogeneity assessment. This was done by visual inspection of gel after the settlement of gel in suitable containers. Gels were analyzed for their appearance and existence of any clog.

**pH Evaluation:**

The pH of the gel was recorded using digital pH meter (Beckman, Germany). It was done by bringing the gel in direct contact with pH meter. After this, pH meter was allowed to equilibrate, then pH is recorded. All the experiments were performed

**Skin irritation test:**

This test was performed on human volunteers. Twenty volunteers were chosen for single formulation and study was performed after taking their informed consent. It was performed by applying gel on an area of 2 square inch to the back of hand. Then the examination for the presence of lesion or irritation was done.

**Spreadability:**

To measure the spreadability of the gels wooden block and glass slide apparatus was used. Approximately, 2 g of developed methotrexte gel was placed in the pan. The time taken by upper slide to separate completely from the fixed slides was noted. The gel spreadability was evaluated through following equation:

**S = ML/T** ….. Eq. 2

Where,

S = spreadability,

M = weight tied to upper slide,

L = length of glass slide, and

T = time taken by the slide to separate from

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**Gelation temperature:**

Evaluation of gelation temperature was done by placing a thin walled tube (containing 2 ml of MTX topical gel) in thermostatically regulated water bath and the temperature of which was increased at consistent rate of 2°C/5 minutes with mild shaking at periodic intervals till it was transformed into the gel. Gelation temperature at which it was distinguished from "flow liquid sol" to "no flow solid gel" upon the inversion of tube was considered to be the gelation temperature of the sample.

**Gelation time:**

Gelation time is defined as the time taken for conversion of sol to gel. When the optimized formulation was place at gelation temperature. Test tube inverting technique was used to find out the gelation time which involves the use of thin-walled glass tube (containing 2 ml of MTX topical gels) placed in experiment was performed at static temperature by using controlled water bath at the gelation temperature (earlier determined) with gentle shaking at periodic intervals. The time (second) necessary for conversion to gel is recorded as gelation time which was evaluated by flow or no-flow condition when the test tube was inverted.

**Drug content of gel:**

A measured amount of formulated gel was taken and dissolved in 100 ml of ethanolic phosphate buffer of pH 7.4. Similarly, solution of marketed formulation of matrexate was also prepared. Mechanical shaker was used to shake the gel solution continuously for 2 hours. The solution thus prepared was filtered and analyzed spectrophotometrically at 303 nm using ethanolic buffer (pH 7.4) as blank.

The drug content of topical gel formulation in phosphate buffer pH 7.4, calculated by using the formula: Entrapment efficiency= [drug content (experimental)/drug content (theoretical)]*100

 Viscosity study:

The viscosity of optimized formulation was determined by using Brookfield viscometer (DV-II+, Brookfield, USA). The viscosity measurements were conducted by using suitable spindle (no. 8) at appropriate speed (30 rpm). [170] Thermo-stated water jacket was used to record viscosity (n = 3) at two temperatures 8°C ± 1°C and at 37°C ± 1°C. The samples were equilibrated for 10 minutes previous to measurement; also, the instrument was equipped with a temperature control unit.

**. *In-vitro* drug release studies:**

The Cellophane membrane membrane was taken, and it was tied to one end of the open (diffusion) tube and slightly immersed in the receptor medium comprising of 30 ml of pH 7.4 phosphate buffer in 100 ml Borosil beaker; 1 ml of topical gels was placed within Cellophane membrane, which acted as the donor compartment.

The receptor medium was maintained at 37°C ± 0.5°C, stirred at 100 rpm. At normal intervals, a 3 ml of diffusion medium was withdrawn from the receptor compartment and replaced with equal volume of fresh warm buffer solution. The drug concentration was determined using UV-Visible spectrophotometer at wavelength 303 nm. [184][170] Formula for determination of percentage of release of drug from in vitro dissolution testing:

Concentration of drug (µg/ml) = (slope × absorbance) ± intercept

Amount of drug released mg/ ml = Concentration × Dissolution bath volume × dilution factor/1000. Cumulative percentage release (%) = Volume of sample withdrawn (ml)/bath volume (v) × P (t – 1) + Pt Where,

Pt = Percentage release at time t Where,

P (t – 1) = Percentage release previous to „t"

## 4. RESULTS AND DISCUSSION:

**Preformulation study of Methotrexate:**

**Physical examination of drug**: Physical properties of methotrexate drug were found to be slightly orange crystalline powder

**Melting point**: The melting point of Methotrexate was determined by melting point apparatus and found to be 182-188ºC.

**Preparation of Standard Curve:**

Topical gels of Methotrexate were prepared by "Cold technique" as previously described. Methotrexate topical gels were formulated using Poloxamer (20% to 24%) as thermo sensitive polymer and tamarind seed gum (0.3%–0.9%) and characterization were carried out to assess their properties. The λmax of Methotrexate drug was found to be 303nm in phosphate buffer 7.4pH. The Regressed equation is shown below:-

$y = 0.056x + 0.008$, $R^2 = 0.998$ [10]

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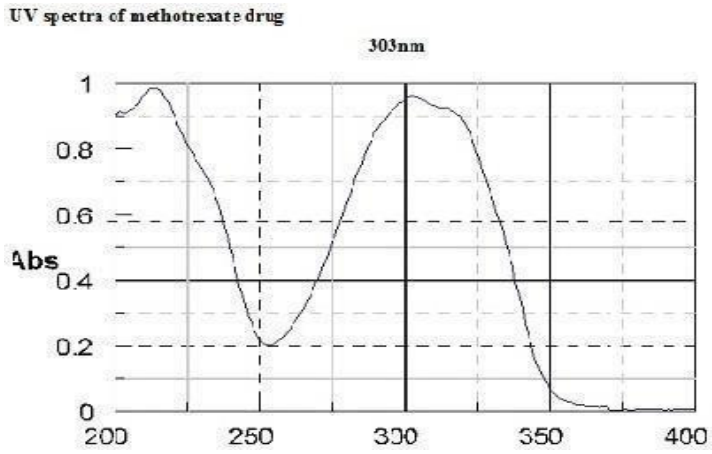
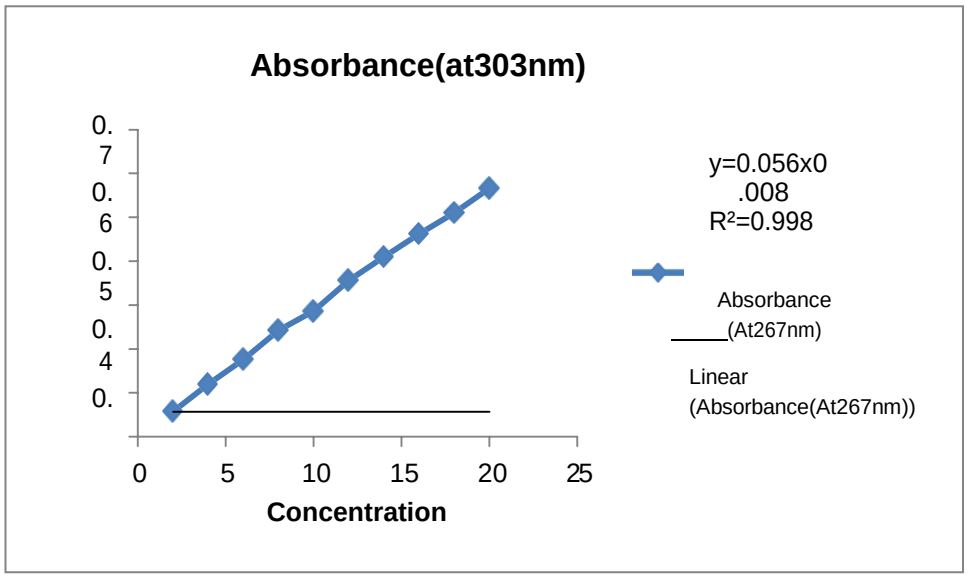



**Fig 1: λmax of Methotrexate at 7.4 pH at 303 nm**

**Table 2 Data for**
**Calibration curve of Methotrexate in PBS 7**.

| S. No | Concentration(µg/ ml) | Absorbance(At267nm) | Regressed value |
|---|---|---|---|
| 1. | 2 | 0.0578 | 0.12 |
| 2. | 4 | 0.11995 | 0.232 |
| 3. | 6 | 0.17668 | 0.344 |
| 4. | 8 | 0.24272 | 0.456 |
| 5. | 10 | 0.28671 | 0.568 |
| 6. | 12 | 0.35701 | 0.680 |
| 7. | 14 | 0.4101 | 0.792 |
| 8. | 16 | 0.4628 | 0.904 |
| 9. | 18 | 0.5108 | 1.016 |
| 10. | 20 | 0.5669 | 1.128 |




•Distribution coefficient: The distribution behavior of drug is examined in n-octanol: water and n-octanol: PBS (pH 7.4) systems by using UV method. The distribution coefficient of drug was found to be 0.32.

Table: 3. Distribution coefficient of Methotrexate in different solvents.

| S.No. | Phases | Result |
|---|---|---|
| 1. | n-Octanol/Phosphate buffer saline(pH7.4) | 0.312 |
| 2. | n-Octanol/Water | 0.281 |

**Compatibility studies:**

MTX and their topical gels formulation (F8) were analyzed for compatibility testing by using FT-IR to find out any interactions between the polymers and the drug used. The FT-IR spectra of MTX, tamarind seed gum, pluronic, and its physical mixture were determined. The functional groups with corresponding peaks of pure MTX and in physical mixture of MTX and polymers were found to be correlative, and no any other peak found. The main peaks of MTX, i.e., 3,363.97 cm−1 to N-H stretching; 1,645.33 cm−1 to C=O stretch; 1,448.59 cm−1 to CH deformation (CH3); and 831.35 cm−1 to C-H deformation (aromatic) were found in the FT-IR spectra of physical mixture as shown in Figure. The FT-IR spectra of the drug, with other co-polymers where the entire characteristic peaks of MTX and there was no change in their position, representing no chemical interactions between them. These results established the compatibility of drug and polymer(s) used.

**Evaluation:**

**Thermo sensitivity characterization:**

 Gelation temperature:

The gelation temperature of Methotrexate topical gels were used 29.6°C ± 0.28°C to 38.3°C ± 0.43°C. Out of eight topical gels, two formulations (F1 and F3) gelled at a temperature little above 37°C. Optimized formulation F8 showed the lowest gelation temperature of 27.2±0.18°C and F1 the maximum gelation temperature of 38.3°C ± 0.42°C (Table 2). Remaining formulations got transformed to gel below of the body temperature. The presence of co- polymer considerably altered the process of poloxamer gelation process. In the study, incorporation of tamarind seed gum (0.3%– 0.9% w/v) showed noticeable effect on the gelation temperature, which was concentration dependant.

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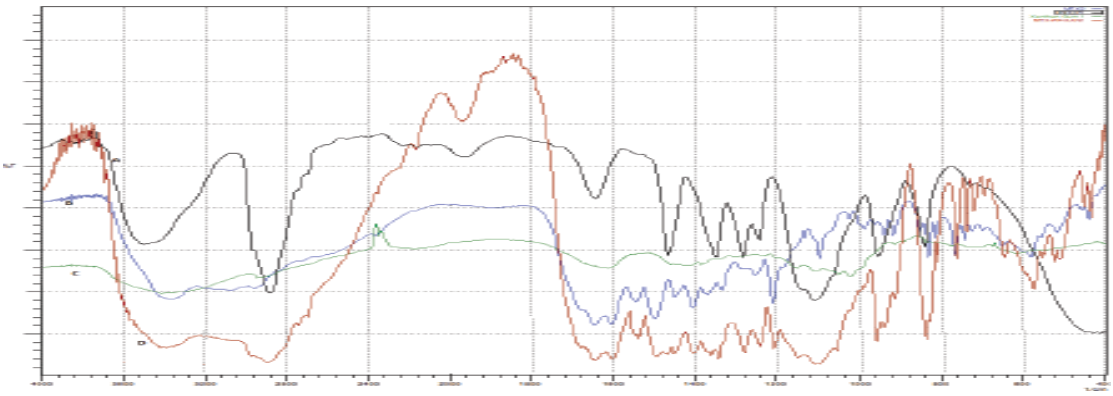

Fig 7.3: FT-IR spectra of Methotrexate (A),

Tamarind seed gum (B), Poloxamer (C), and Physical Mixture (D).

**In-vitro drug release study:** The results obtained from in vitro diffusion studies inferred that drug release was in a sustained manner (Fig. 4.). Gelation of poloxamer occurs as a result of dehydration of the polymer, the Pluronic micelles come in contact with one another causing the entanglements among the hydrophilic corona of PEO chains, and thus the gel structure is formed. Due to the increase in the temperature, it can form hexagonal-packed cylindrical micelles, which stack together, leading to decreased release rates

**Table:4 In-vitro % Cumulative drug release rate:**

| Mins | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 22.05±0.12 | 27.21±0.18 | 27.67±0.72 | 22.58±0.27 | 18.8±0.34 | 23.13±0.20 | 24.03±0.02 | 25.0±0.22 |
| 20 | 47.51±0.41 | 46.6±0.24 | 47.7±0.17 | 29.53±0.35 | 33.85±0.50 | 38.11±0.32 | 40.12±0.21 | 43.15±0.51 |
| 40 | 65.81±0.19 | 67.14±0.57 | 51.98±0.15 | 50.44±0.41 | 54.13±0.23 | 69.21±0.21 | 69.1±0.11 | 71.2±0.20 |
| 60 | 70.84±0.21 | 78.79±0.19 | 71.80±0.44 | 69.7±0.33 | 67.21±0.61 | 77.67±0.44 | 78.55±0.21 | 80.95±0.57 |
| 80 | 82.16±0.61 | 87.53±0.35 | 84.5±0.32 | 81.78±0.40 | 78.33±0.70 | 84.3±0.32 | 86.7±0.33 | 88.4±0.47 |
| 100 | 86.46±0.40 | 89.31±0.13 | 88.20±0.38 | 84.78±0.40 | 87.65±0.53 | 90.4±0.47 | 91.6±0.32 | 92.5±0.58 |
| 120 | 87.05±0.61 | 90.31±0.13 | 89.40±0.29 | 85.78±0.40 | 88.14±0.57 | 92.30±0.65 | 95.10±0.20 | 96.65±0.60 |

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**Table: 5. Result and Characteristics of prepared Methotrexate topical gels**

| Formula-code | Appear-nce | Gelationtempera-ture | Gelationteme (insec.) | Drugcont-ent % | Viscosity(cps) | |
|---|---|---|---|---|---|---|
| | | | | | $8^0$C | $37^0$C |
| F1 | +++ | 38.2±0.20 | 55±2 | 98.71±0 | 523±2 | 25703±1 |
| | | | | .72 | 0 | 33 |
| F2 | +++ | 35.9±0.67 | 45±1 | 97.69±0 | 1503± | 452387± |
| | | | | .50 | 11 | 348 |
| F3 | +++ | 37.5±0.12 | 40±1 | 96.51±0 | 1813± | 55236±4 |
| | | | | .11 | 46 | 21 |
| F4 | +++ | 36.8±0.56 | 35±1 | 97.88±0 | 1742± | 45260±3 |
| | | | | .34 | 28 | 26 |
| F5 | +++ | 34.7±0.31 | 34±2 | 98.77±0 | 1823± | 58455±4 |
| | | | | .23 | 53 | 02 |
| F6 | +++ | 27.2±0.18 | 29±2 | 98.10±0 | 2101± | 60141±3 |
| | | | | .90 | 11 | 40 |
| F7 | +++ | 33.2±0.80 | 33±2 | 97.67±0 | 2056± | 67461±5 |
| | | | | .22 | 78 | 32 |
| F8 | +++ | 28.3±0.22 | 30±2 | 98.13±0 | 2203± | 68243±5 |
| | | | | .20 | 34 | 20 |

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## 5. CONCLUSION

The presence of co-polymer considerably altered the process of poloxamer gelation process. In the study, incorporation of tamarind seed gum (0.3%–0.9% w/v) showed noticeable effect on the gelation temperature, which was concentration dependant. At high temperature or at high strength, these micelles relate to form different lyotropic isotropic liquid crystalline phases change with enhance in temperature, micellar entanglement proceeds, leading to formation of gel and consequential in the overall amplify of bulk viscosity. With enhance in length of poly (oxyethylene) (PEO) chain the onset and temperature of gelation and thermal stability of the gel also increases. This results in hydrophobic interactions within polyoxypropylene domains and leads to the formation of gel. The gelation time for MTX gels was between 28 ± 2 seconds and 55 ± 2 seconds. As observed, gelation time decreased as the concentration of the co-polymers was increased in the formulation. Longest gelation time of 55±2 in second was recorded with F1, whereas shortest gelation time of 30±2seconds was with F8 decrease of seconds was observed with increase of polymer concentration from 0.3% to 0.9% in formulations F3 to F8. Quick topical gels help in retaining the drug at the site of injection and release in a controlled manner.

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